Cerebrospinal Fluid Exposure of Efavirenz and Its Major Metabolites When Dosed at 400 mg and 600 mg Once Daily: A Randomized Controlled Trial

Alan Winston,¹ Janaki Amin,² Amanda Clarke,³ Laura Else,⁴ Alieu Amara,⁴ Andrew Owen,⁴ Tristan Barber,⁵ Heiko Jessen,⁶ Anchalee Avinghsanon,⁷ Ploenchon Chetchotisakd,⁷ Saye Khoo,⁴ David A. Cooper,² Sean Emery,² and Rebekah Puls²; for the ENCORE Cerebrospinal Fluid (CSF) Substudy Team

¹Section of Infectious Diseases, Imperial College London, United Kingdom; ²Kirby Institute, University of New South Wales, Sydney, Australia; ³Thai Red Cross AIDS Research Centre, Bangkok, Thailand; ⁴Department of Pharmacology, University of Liverpool, and ⁵Chelsea and Westminster NHS Foundation Trust, London, United Kingdom; ⁶Medical Group Practice, Berlin, Germany; and ⁷Srinagarind Hospital, Khon Kaen University, Thailand

Background. The optimal penetration of antiretroviral agents into the central nervous system may be a balance between providing adequate drug exposure to inhibit human immunodeficiency virus (HIV) replication while avoiding concentrations associated with neuronal toxicities.

Methods. Cerebrospinal fluid (CSF) exposure of efavirenz and the metabolites 7-hydroxy (7OH) and 8-hydroxy (8OH) efavirenz were assessed after at least 12 weeks of therapy in HIV-infected subjects randomized to commence antiretroviral regimens containing efavirenz at either 400 mg or 600 mg once daily.

Results. Of 28 subjects (14 and 14 on efavirenz 400 mg and 600 mg, respectively), CSF HIV RNA was undetectable in all. Geometric mean CSF efavirenz, 7OH-, and 8OH-efavirenz concentrations (with 90% confidence intervals [CIs]) for the 400-mg and 600-mg dosing groups were 16.5 (13–21) and 19.5 (15–25) ng/mL; 0.6 (.4–.9) and 0.6 (.4–1) ng/mL; and 5.1 (4.0–6.4) and 3.1 (2.1–4.4) ng/mL, respectively. Efavirenz concentration in CSF was >0.51 ng/mL (proposed CSF 50% maximal inhibitory concentration for wild-type virus) in all subjects, and 8OH-efavirenz concentration in CSF was >3.3 ng/mL (a proposed toxicity threshold) in 11 of 14 and 7 of 14 subjects randomized to the 400 mg and 600 mg doses of efavirenz, respectively. Whereas CSF efavirenz concentration was significantly associated with plasma concentration (P < .001) and cytochrome P450 2B6 genotype (CSF efavirenz GG to GT/TT geometric mean ratio, 0.56 [90% CI, .42–.74]), CSF 8OH-efavirenz concentration was not (P = .242 for association and CSF 8OH-efavirenz GG to GT/TT geometric mean ratio, 1.52 [90% CI, .97–2.36]).

Conclusions. With both doses of efavirenz studied, CSF concentrations were considered adequate to inhibit HIV replication, although concentrations of 8OH-efavirenz were greater than those reportedly associated with neuronal toxicity. CSF exposure of 8OH-efavirenz was not dependent on plasma exposure and, as we postulate, may be subject to saturable pharmacokinetic effects.

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Keywords. efavirenz; pharmacokinetics; pharmacogenomics; CSF; HIV.

Sanctuary site exposure of antiretroviral agents may be of clinical relevance, with the central nervous system (CNS) being a sanctuary site of key interest. Higher concentrations of antiretroviral drugs in the CNS may be associated with improved human immunodeficiency virus (HIV) virological suppression [1, 2] in the brain compartment. It has been postulated this...
may be associated with improved clinical outcomes, such as cognitive function [3]. Conversely, greater penetration of antiretroviral agents may be associated with an increased propensity for drug-related CNS toxicities [4].

The use of antiretroviral agents at doses lower than those currently licensed may offer several benefits including reduced health-care costs and the potential to reduce drug-associated toxicities. Recently, an antiretroviral regimen containing efavirenz (EFV) dosed at 400 mg once daily rather than the currently approved dose of 600 mg once daily has shown virological noninferiority within a randomized controlled study [5]. The CNS exposure of EFV at this lower dose has not previously been determined.

EFV is metabolized by the cytochrome P450 (CYP) isoenzyme to several products, including 8-hydroxy efavirenz (8OH-EFV), the major metabolite via CYP2B6 [6], and, to a lesser extent, to 7-hydroxy efavirenz (7OH-EFV) via CYP2A6 [7]. Although these metabolites are reported to be inactive in terms of antiviral activity [8], they may be associated with toxicities. Potential CNS toxicities are reported to be associated with 8OH-EFV [9].

The aim of this study was to assess the cerebrospinal fluid (CSF) exposure, as a surrogate for CNS exposure, of EFV and its major metabolites when dosed at 400 mg and 600 mg once daily in HIV-infected subjects after at least 12 weeks of commencing initial antiretroviral therapy. Furthermore, we assessed the number of subjects with EFV CSF exposure above concentrations expected to be associated with virological efficacies. Potential CNS toxicities are reported to be associated with 8OH-EFV [9].

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METHODS

Subject Selection

Antiretroviral-naive, HIV-infected adults entering the ENCORE1 study (A randomized, double-blind, placebo-controlled, clinical trial to compare the safety and efficacy of reduced-dose EFV with standard-dose EFV plus tenofovir and emtricitabine in antiretroviral-naive HIV-infected individuals over 96 weeks; see http://clinicaltrials.gov/ct2/show/NCT01011413 [5]) were eligible to enter this subStudy Procedures

Between 12 and 24 weeks of commencing study therapy, CSF examination was undertaken at least 8 hours after dosing of study medication. General safety assessments, including cerebral imaging and assessment of blood clotting, were undertaken as per usual clinical practice at each site prior to undertaking a lumbar puncture examination. The following analyses on CSF samples were undertaken: total protein; assessment of EFV, 8OH-EFV, and 7OH-EFV; and CSF HIV RNA level.

Blood sampling immediately prior to CSF examination was undertaken to assess plasma EFV, 8OH-EFV, and 7OH-EFV concentrations.

Pharmacokinetic, Pharmacogenomic, and Ultrasensitive HIV RNA Analysis

Concentrations of total (conjugated and unconjugated to plasma proteins) EFV and its metabolites in plasma and CSF were analyzed by high-performance liquid chromatography–tandem mass spectrometry by methods previously described [11]. In brief, freshly prepared standards and quality control samples (prepared in artificial CSF) and clinical samples (100 µL) were incubated at 37°C for 2 hours with 400 µL of a solution containing 200 units of β-glucuronidase from Helix pomatia in 0.2 M sodium acetate buffer (pH = 5). An internal standard (hexobarbital) was added and samples were subsequently alkalized with 20 µL of potassium carbonate buffer (0.1 M, pH = 9.4) and then extracted with 3 mL of ethylacetate:hexane (60:40 v/v). After centrifugation, the organic phase was evaporated to dryness and the residue reconstituted in 100 µL of mobile phase (50/50 v/v acetonitrile/ [ACN]/water [H2O] in 1 mM ammonium acetate). Quantification was performed using a Thermo Vantage Triple Quadrupole mass spectrometer using selective reaction monitoring in negative ionization mode. The lower limit of quantitation (LLOQ) for plasma EFV, 8OH-EFV, and 7OH-EFV and CSF EFV, 8OH-EFV, and 7OH-EFV was 25, 5, 5, 2.5, 1.25, and 0.625 ng/mL, respectively. Inter- and intra-assay precision did not exceed 10% for any compound. The CYP2B6 516G→T genetic polymorphism was identified by real-time polymerase chain reaction (PCR) [12, 13].

Quantification of CSF HIV RNA was performed using a highly sensitivity in-house assay with a detection limit of 2 RNA copies/mL, assuming an available volume of 2 mL. When <2 mL sample was available, the cutoff was adjusted proportionately. In brief, virus was pelleted by centrifugation and RNA extracted by the Qiagen MiniElute method. The eluate was reverse-transcribed and amplified for 20 cycles using the Invitrogen One-Step method, and PCR products were quantified in a nested real-time PCR using the Qiagen Probe PCR method. A standard curve was generated from dilutions of the international working reagent WR1 (National Institute for Biological Standards and Control, Potters Bar, UK) [14].
**RESULTS**

**Subject Characteristics**

Baseline characteristics are summarized in Table 1. Race/ethnicity was similar in both study treatment groups (9, 5, and 1 [400-mg group] and 11, 4, and 1 [600-mg group] subjects of Asian, white, and African race/ethnicity, respectively). Successful CSF examination was achieved in 28 subjects. Lumbar puncture examination was unsuccessful in 1 subject allocated to 400 mg of EFV and 2 subjects allocated to 600 mg of EFV after 2 attempts. Further attempts were not considered clinically appropriate. Lumbar puncture examination was performed after a mean of 22 weeks (range, 11–25 weeks) after commencing study therapy, at which time all subjects remained on randomized study therapy.

Mean CSF protein was 0.40 g/dL (range, 0.24–1.02 g/dL). At the time of the CSF examination, plasma HIV RNA was undetectable in all subjects (<5 copies/mL in all but 1 subject in whom HIV RNA was <10 copies/mL due to a small volume for analysis), and CSF HIV RNA was also undetectable in all subjects (again <5 copies/mL in all but 1 subject, due to CSF volume constraints, the cutoff was 10 copies/mL).

**Pharmacokinetic Results**

Exposure of EFV and its major metabolites in the plasma and CSF are shown in Table 2. Concentrations of EFV in both the plasma and CSF were slightly lower when dosed at 400 mg vs 600 mg, although this was not statistically significant (geometric mean ratio [GMR], 0.76 [90% CI, .53–1.09] and 0.84 [90% CI, .61–1.18] for plasma and CSF, respectively). There were no significant differences in overall CSF to plasma ratio for EFV concentration between the 2 study doses, and CSF EFV concentration was not associated with CSF protein (P = .095 for differences between randomized treatment groups).

A similar trend was observed for 7OH-EFV exposure between the 2 studied doses of EFV. Concentrations of 7OH-EFV in the CSF were below the assay LLOQ of 0.625 ng/mL in 15 subjects (7 and 8 on EFV 400 mg and 600 mg, respectively).

A different trend was observed regarding 8OH-EFV exposure (Figure 1 and Table 2). Plasma exposure of 8OH-EFV was similar when EFV was dosed at 400 mg vs 600 mg (GMR, 1.04 [90% CI, .76–1.43]), and CSF exposure followed a similar pattern, with exposure slightly increased in the 400-mg arm (GMR [400 mg/600 mg], 1.65 [90% CI, 1.09–2.50]). When excluding 1 outlier with a high CSF 8OH-EFV concentration (13 ng/mL), this observation persisted (GMR [400 mg/600 mg], 1.53 [90% CI, 1.01–2.32]).

Concentration of EFV in CSF at 8–16 hours postdose was >0.51 ng/mL in all subjects in both treatment groups, and exposure of 8OH-EFV in the CSF was >3.3 ng/mL in 11 of 14 and 7 of 14 subjects allocated to the 400 mg and 600 mg doses of EFV, respectively (P = .095 for differences between randomized treatment groups).

**Factors Associated With CSF 8OH-Efavirenz Exposure**

As we observed a trend toward higher exposure of 8OH-EFV in the CSF with the lower dose of EFV studied, we undertook
exploratory analyses to assess pharmacokinetic and clinical factors associated with the exposure of this metabolite.

Although CSF EFV exposure was associated with plasma EFV exposure (Spearman correlation coefficient 0.70, \( P < .001 \)), CSF 8OH-EFV exposure was not significantly associated with either plasma EFV or plasma 8OH-EFV exposure (Spearman correlation coefficient \(-2.3 \) and \(0.08, P = .242 \) and \( .674, \) respectively). Pharmacogenomic associations between CSF EFV and its metabolites are shown in Table 3. As expected, higher CSF EFV exposure was observed in subjects with GT or TT genotypes in CYP2B6. However, no association was observed between CSF 8OH-EFV concentration and the CYP2B6 genotypes tested. Interestingly, the ratio of CSF 8OH-EFV to CSF EFV exposure was lower in subjects with GT or TT genotypes in CYP2B6; this effect was not observed for the ratio of CSF 7OH-EFV to CSF EFV.

Exposure of CSF 8OH-EFV was significantly associated with a variety of patient reported outcomes from the main ENCORE1 study including ESQ results at weeks 4 and 48 (Table 4).

**DISCUSSION**

We have made several interesting observations when assessing the CSF exposure of EFV and its major metabolites in virologically suppressed adult patients dosed at 400 mg and 600 mg once daily. As expected, the CSF exposure of EFV was slightly

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*Figure 1.* Mean plasma and cerebrospinal fluid (CSF) concentrations of 8-hydroxy (8OH) efavirenz. Circle and triangles denote mean concentrations; lines denote 90% confidence intervals.
lower when EFV was dosed at 400 mg vs 600 mg daily. However, CSF exposure of the 8OH-EFV metabolite was similar with both dosing schedules, with a trend toward higher concentrations observed with the 400-mg dosing schedule. Concentrations of EFV in CSF remained above 0.51 ng/mL, a postulated threshold observed with the 400-mg dosing schedule. Concentrations of both dosing schedules, with a trend toward higher concentration in subjects with a slower EFV metabolism genotype and, as expected, higher CSF EFV exposure was observed in subjects with GT or TT genotypes in CYP2B6. Therefore, in subjects with a slower EFV metabolism genotype, plasma exposure of EFV is higher compared to those with faster EFV metabolism genotype and, as expected, CSF exposure is also higher.

However, such a signal was not observed for CSF 8OH-EFV exposure, where CSF concentrations were similar between subjects with GG and in those with GT or TT genotypes in CYP2B6. Also, the ratio of CSF 8OH-EFV to CSF EFV exposure was statistically significantly lower in subjects with GT or TT genotypes, but this effect was not observed with the 7OH-EFV metabolite. We postulate that the underlying pharmacokinetic processes associated with CSF 8OH-EFV exposure may be subject to a saturation effect whereby exposure to this metabolite is not dependent on plasma EFV exposure or CYP2B6 genotype (both of which we have observed), but is rather a spillover from plasma 8OH-EFV or local CNS production of 8OH-EFV whereby this metabolite becomes “trapped” within the CNS compartment. A possible saturable process may be glucuronidation of 8OH-EFV in the CNS compartment whereby this saturated compound is unable to cross the blood–brain barrier; within the different EFV dosing schedules studied in our trial, the exposure of the 8OH-EFV metabolite in the CSF was independent of EFV dose, plasma exposure, and CYP2B6 genotype.

Table 3. Pharmacogenomic Associations With Cerebrospinal Fluid Efavirenz Exposure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Genotype</th>
<th>Geometric Mean</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF EFV, ng/mL</td>
<td>GG</td>
<td>14.26</td>
<td>12.37-16.43</td>
</tr>
<tr>
<td></td>
<td>GT/TT</td>
<td>25.48</td>
<td>18.90-34.35</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td>0.56</td>
<td>.42-.74</td>
</tr>
<tr>
<td>CSF 8OH-EFV, ng/mL</td>
<td>GG</td>
<td>4.66</td>
<td>3.66-5.92</td>
</tr>
<tr>
<td></td>
<td>GT/TT</td>
<td>3.07</td>
<td>1.97-4.77</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td>1.52</td>
<td>.97-2.36</td>
</tr>
<tr>
<td>Ratio of CSF 8OH-EFV to CSF EFV</td>
<td>GG</td>
<td>0.33</td>
<td>.26-.41</td>
</tr>
<tr>
<td></td>
<td>GT/TT</td>
<td>0.12</td>
<td>.07-.22</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td>2.71</td>
<td>1.60-4.58</td>
</tr>
<tr>
<td>Ratio of CSF 7OH-EFV to CSF EFV</td>
<td>GG</td>
<td>0.04</td>
<td>.03-.05</td>
</tr>
<tr>
<td></td>
<td>GT/TT</td>
<td>0.03</td>
<td>.02-.06</td>
</tr>
<tr>
<td>Ratio</td>
<td></td>
<td>1.05</td>
<td>.63-1.75</td>
</tr>
</tbody>
</table>

Ratios are GG to GT/TT.

Abbreviations: 7OH, 7-hydroxy; 8OH, 8-hydroxy; CI, confidence interval; CSF, cerebrospinal fluid; EFV, efavirenz.

Table 4. Correlation Between Cerebrospinal Fluid 8-Hydroxy Efavirenz Exposure and Patient-Completed Questionnaires

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week</th>
<th>Spearman Correlation Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASS-Depression</td>
<td>48</td>
<td>0.20</td>
<td>.31</td>
</tr>
<tr>
<td>DASS-Anxiety</td>
<td>48</td>
<td>0.11</td>
<td>.58</td>
</tr>
<tr>
<td>DASS-Stress</td>
<td>48</td>
<td>0.38</td>
<td>.04</td>
</tr>
<tr>
<td>ESQ</td>
<td>4</td>
<td>-0.43</td>
<td>.02</td>
</tr>
<tr>
<td>ESQ</td>
<td>48</td>
<td>0.13</td>
<td>.05</td>
</tr>
<tr>
<td>SF-12-Physical score</td>
<td>48</td>
<td>0.13</td>
<td>.50</td>
</tr>
<tr>
<td>SF-12-Mental score</td>
<td>48</td>
<td>-0.38</td>
<td>.05</td>
</tr>
</tbody>
</table>

Abbreviations: DASS, Depression Anxiety Stress Scales; ESQ, efavirenz symptom questionnaire; SF-12, 12-item short form.
A further explanation for our findings could be secondary to the effects EFV has on CYP2B6 induction. EFV is extensively metabolized by the CYP2B6 isoenzyme group, mainly to the 8OH-EFV metabolite, and is also an autoinducer of its own metabolism via induction of CYP2B6 [22]. The CYP2B6 isoenzymes are present not only in hepatic tissue but also in the blood–brain barrier [23], raising the possibility for local EFV metabolism within the CNS compartment. If EFV autoinduction was dose proportionate, then with higher doses of EFV and greater autoinduction, greater concentrations of the 8OH metabolite would be expected. However this is not in keeping with our observations. Conversely, if the effects of EFV on autoinduction are not dose proportionate, and could even be greater with lower doses or vary in anatomical locations with varying doses, increased induction of CYP2B6 could lead to increased concentrations of metabolites produced via this pathway, such as the 8OH-EFV metabolite, even when a lower dose of the parent drug is administered. However, one would expect an autoinduction effect for a specific dose of a drug to be similar between subjects with faster and slower metabolism genotypes; we did not observe this effect in our study and therefore do not think a differing effect on autoinduction is a plausible explanation for our findings.

Antiretroviral drug delivery to the CNS is dependent on several influx and efflux transport proteins expressed at the blood–brain barrier. Changes in the expression of these transporters may alter the exposure of antiretroviral drugs in the CNS, and antiretroviral agents including EFV and metabolites of EFV may themselves alter expression of such transporters [24]. Such effects, if present, may vary with EFV dose. It is possible that with higher doses of EFV, greater expression of efflux transport proteins may occur. An upregulation in efflux transporters that was dependent on EFV dose and particularly affected the removal of 8OH-EFV from the CNS provides another theoretical explanation for our findings.

Within the main ENCORE1 study, no differences in the number of patients reporting adverse events were observed, although study drug–related adverse events were more frequently observed in the 600-mg group than in the 400-mg group [5]. These study drug–related adverse events occurred predominantly early, within the first 12 weeks of the study. We believe our study provides one possible explanation for these findings, whereby the lack of difference in overall patient reported adverse events may be related to the lack of difference in 8OH-EFV exposure in both the plasma and CSF compartments with the different doses of EFV studied.

The ideal dose of EFV may be one that, on the one hand, maintains adequate plasma and sanctuary site exposure to induce and maintain an effective virological response, yet also limits exposure in sanctuary sites, including the CNS, to concentrations below those associated with toxicity. Our study suggests that although 400 mg of EFV is associated with CSF exposure of EFV above the concentration required to suppress HIV replication, exposure of metabolites may still be within the concentration range associated with toxicities.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


APPENDIX

Project team: Janaki Amin, Dianne Carey, Kymme Courtney-Vega, Carlo Dazo, Anna Donaldson, Sean Emery, Natalie Espinosa, Peeraporn Kaew-on, Praphan Phanupak, Rebekah Puls, Kanitta Pussadee, Louise Tomkins, Sasiwimol Ubolyam.

Site staff: Thai Red Cross AIDS Research Centre, Bangkok, Thailand; Praphan Phanuphak, Amanda Clarke, Anchalee Avinthsanon, Peeraporn Kaew-on, Kanitta Pussadee, Louise Tomkins, Sasiwimol Ubolyam. Khon Kaen University Hospital, Thailand; Ploenchon Chetchotisakd. Medical Group Practice, Berlin, Germany; Heiko Jessen, Carmen Zedlack. Chelsea and Westminster Hospital, London; Brian Gazzard, Tristan Barber, Serge Federle, Sophie Scott. St Mary’s Hospital, London; Alan Winston, Borja Mora-Peris, Ken Legg, Scott Mullaney.

Laboratory: University of Liverpool, United Kingdom; Laura Else, Alieu Amara, David Back, Sujan Dilly Penchala. Imperial College London; Steve Kaye. BioBank, HIV Immunovirology Research Laboratory, St Vincent’s Centre for Applied Medical Research, Sydney, Australia; Tony Kelleher, Philip Cunningham, Kate Merlin, Julie Yeung, Ansari Shaik, Bertha Fsadni, Alex Carrera, Melanie Lograsso.